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Soybean Lectin and Related Proteins in Seeds and Roots of Le^+ and Le^- Soybean Varieties[1]

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ABSTRACT

The localizations of soybean lectin (SBL) and antigenically related proteins in cotyledons and roots of lectin positive (Le^+) and lectin negative (Le^-) soybean cultivars were compared by light level immunocytochemistry using antibodies produced against the 120 kilodalton (kD) native seed lectin tetramer or its subunits. Lectin is present in the protein bodies of cotyledons cells as are two other seed proteins, the Kunitz trypsin inhibitor and the storage protein glycinin. Analysis of single seed extracts by immunoblotting of sodium dodecyl sulfate-polyacrylamide gels using the same antibodies, reveals up to 4 milligrams of the 30 kD seed lectin protein is present per seed in the Le^+ varieties. There is no detectable lectin in the protein bodies of Le^- cotyledons as determined by immunocytochemistry and immunoblotting. Enzyme-linked immunosorbent assay confirmed this result to a sensitivity of less than 20 nanograms per seed. In contrast, the roots of both Le^+ and Le^- plants bind the seed lectin antibody during immunocytochemistry, with fluorescence mainly localized in vacuole-like bodies in the epidermis. Root extracts contain a 33 kD polypeptide that binds anti-SBL antibody at an estimated minimal level of 20 nanograms per 4-day seedling, or 2.0 nanograms per primary root tip. This polypeptide is also present in the embryo axis and in leaves. The latter also contain a 26 kD species that binds seed lectin antibody. The 30 kD seed lectin subunit, however, is not detectable in roots or leaves.

Lectins are carbohydrate-binding proteins found at moderately high levels in the seeds of many plants, including legumes such as pea, clover, and soybean. The well-characterized agglutinin of soybean (SBL)[2] is a 120,000 D tetrameric glycoprotein having affinity for GalNAc (16). Evidence indicates that it is the product of a single gene locus (19). Furthermore, some soybean cultivars do not produce seed lectin because a transposable element insertion into this gene blocks its expression (10, 31). The presence of lectins in other parts of the soybean plant, such as roots, has been the subject of several reports (8, 9, 21, 26) but their relationships to seed lectin is still unclear. This information is important because of the potential involvement of SBL or other lectins in the attachment of *Rhizobia* to legume roots (see Dazzo and Gardiol [4] for review). Furthermore, the function of non-seed lectins found in various organs and tissues (*e.g.* stems, leaves, roots, bark) (1, 6, 17, 25, 27) is also of interest.

In the present paper, we have compared the localization of lectin in the cotyledons of Le^+ and Le^- soybean varieties using antibodies generated against SBL. The distribution of two other seed proteins, KTi and glycinin storage protein were also examined. All three proteins were localized within protein bodies and lectin was absent from the protein bodies of Le^- varieties. We then probed for the presence of antigenically similar proteins in the roots. Root tip epidermal cells of both Le^+ and Le^- cultivars contained material that cross-reacted with the seed lectin antibody. Immunoblotting of extracts separated by PAGE (Western blotting) was used to characterize and quantitate lectin and cross-reacting proteins. A 33 kD polypeptide was present in the roots and leaves and a 26 kD species was found in leaves. The 30 kD SBL subunit polypeptide was present exclusively in the seed cotyledons and axes and was not found in other plant tissues examined.

MATERIALS AND METHODS

Plant Material. The soybean varieties used were Williams, an Le^+ cultivar, and three Le^- lines, Sooty, PI 90768, and PI 171428 obtained originally from Richard Bernard, curator of the USDA soybean germplasm collection in Urbana, IL. The purity of the Le^- cultivars was assessed by assaying extracts of cotyledon pieces from individual seed in Ouchterlony immunodiffusion plates using antibody to seed lectin. The seed were saved and grown either in the greenhouse or field to maintain the lines. Seed were soaked overnight or germinated for 4 d in moist, rolled papers in the dark at 30°C and then extracted as described below for immunocytochemistry, ELISA, or Western blot analysis.

Preparation of Antibodies. Monospecific antibodies to SBL and KTi were prepared from rabbit sera as described previously (30) by affinity purifying the antibodies on lectin-Sepharose or trypsin inhibitor-Sepharose columns. Antibodies were made by challenging the rabbits with either native protein or denatured antigens treated with 0.1% SDS. Glycinin (11S) protein was purified from soybean seed by the methods of Moreira *et al.* (18). Antibodies were made to glycinin in the same manner as for SBL and purified on a glycinin-Sepharose column. Proteins were coupled to Sepharose 4B by standard procedures (Pharmacia Technical Bulletin, 'Affinity Chromatography').

Tissue Fixation and Immunofluorescence. Cotyledons obtained from imbibed seed or from 5 mm root tips of 4-d seedlings were fixed overnight at 4°C in solution containing freshly prepared 4% formaldehyde (Polysciences, Inc., Warrington, PA) and 0.3 M sucrose in phosphate-buffered saline (PBS; 10 mM sodium phosphate, 123 mM NaCl [pH 7.2]). Samples were extensively washed in PBS at RT. Frozen sections were prepared as previ-

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[2] Abbreviations: SBL, soybean seed lectin; Le^+, seed lectin positive; Le^-, seed lectin negative; KTi, Kunitz trypsin inhibitor; GalNac, *N*-acetylgalactosamine; ELISA, enzyme-linked immunosorbent assay; CRM, cross-reacting material; RT, room temperature.

ously described (17, 24) and placed on slides precoated with 0.1% gelatin and 0.01% chromium-potassium sulfate. Sections were air dried, treated with 0.5% Triton X-100 for 30 min at RT, and then washed extensively in PBS containing 10 mM glycine. The sections were next treated with undiluted nonimmune goat serum for 15 min at RT, after which the serum was drawn off and 300 μl of specific antibody or nonimmune rabbit IgG (each at 60 μg/ml, in PBS containing 10% goat serum and 0.025% Triton) were applied. The sections were incubated overnight at 4°C and washed for a total of 30 min with three changes of PBS plus glycine. After goat serum was applied as before, the sections were incubated for 1 h at RT in rhodamine-conjugated goat anti-rabbit immunoglobulin containing 10% goat serum and 0.025% Triton. Sections were washed as described above, mounted on glass slides, and then viewed with epifluorescence and phase contrast optics (Reichert Zetopan, American Optical Corp., Buffalo, NY). Images were enhanced with a triple intensified vidicon camera (model 9003, Venus Scientific, Farmingdale, NY) and exposures were taken from a video monitor (model 634, Tektronix, Inc., Beaverton, OR) using a 35 mm camera (model FM, Nikon) and Kodak Plus X film as previously described (20).

Preparation of Extracts, Western Blotting, and ELISA. Tissues were macerated in a mortar and pestle with sand in a buffer of PBS plus 10 mM 2-mercaptoethanol. The cotyledons and embryo axis from a single seed were dissected and extracted separately in a total of 5 and 1 ml of buffer, respectively. The extracts were clarified by centrifugation for 15 min at 17,000g at 4°C. Primary root tips of 1 cm in length were dissected from 4-d old seedlings, pooled, frozen in liquid N_2, and dried by lyophilization. Thirty mg of dried root tips were ground in 600 μl of buffer with sand in a mortar and pestle. The extract was then centrifuged in a microtube for 15 min at 15,000g and the supernatant saved. In some cases the pellet resulting from this initial centrifugation was resuspended in PBS containing 10 mM mercaptoethanol and 1% SDS and boiled for 10 min. The denatured extract was centrifuged as before and the supernatant retained for assay. Thirty mg of freeze-dried leaves from adult plants were treated and extracted in a similar manner.

Proteins were separated by SDS-PAGE (15) and blotted to nitrocellulose using an electroblotting apparatus (EC Corporation, St. Petersburg, FL). SBL or cross-reacting proteins were detected immunochemically using anti-SBL and an alkaline phosphatase probe essentially as described by Vierstra *et al.* (29). After blotting, the nitrocellulose filter was rinsed with distilled H_2O and incubated with 10 ml of saturation buffer (3% gelatin, 1% BSA in TBS [pH 9.0]; TBS = 20 mM Tris, 180 mM NaCl [pH 7.5]) or BLOTTO (5% nonfat milk proteins in TBS [pH 7.5]; 13) for 5 to 24 h in a sealable plastic pouch. The blot was rinsed briefly with TBS and monospecific anti-SBL antibody from rabbit was added in BLOTTO to 1.5 μg/ml and the blot incubated in a plastic pouch on a rotator for 5 to 24 h at 4°C. The blot was rinsed after this and subsequent anibody steps with three changes each of 150 ml of BLOTTO. The secondary antibody, alkaline phosphatase conjugated goat anti-rabbit IgG (Sigma A-802) was added at a 1/1000 dilution (1 μg/ml in 10 ml) and incubated for 5 h or overnight and then rinsed with BLOTTO as above and then briefly with TBS. A solution for color development was prepared not more than 30 min before use by dissolving 15 mg of nitro blue tetrozolium in 80 ml of H_2O and 20 ml of 5X color buffer (500 mM Tris, 500 mM NaCl, 25 mM $MgCl_2$ [pH 9.0]). Just before use, 7.5 mg of 5-bromo-4-chloro-3-indoyl phosphate is added. The 5-bromo-4-chloro-3-indoyl phosphate was first dissolved in 100 μl of DMSO in a microtube to which 100 μl of 1 M Tris (pH 7.5) is added. Color development was allowed to proceed for 15 min in the dark with gentle shaking in a glass dish. The blots were rinsed well with distilled H_2O, air dried, and stored in the dark. SBL standard concentrations of 0.3, 0.6, 1.2, 10, 20, 40, 60, 80, and 100 ng were subjected to the same procedures and the band intensities compared to those of extracts in order to quantitate immunobinding. SBL was clearly detectable to limits of 0.6 ng. Since 20 μl of extract were generally applied to the gel, the sensitivity of the Western blot is 30 ng/ml.

A double antibody sandwich ELISA (32) was also used to quantitate SBL. Affinity purified anti-SBL prepared in goat (Vector Laboratories, Burlingame, CA) was bound to the plate at 1 μg/ml as the capture antibody in 0.05 M Na borate (pH 8.9) for 2 h at RT or overnight at 4°C. The antibody was removed, rinsed, and 250 μl of 0.2% BSA in PBS was added for 2 h to block remaining sites on the plates. Three rinses between each step were made with PBS-0.05% Tween 20 and incubations were done at RT. A 50 to 100 μl volume of SBL antigen or extract was added and serially diluted 1:2 in the wells. After incubation for 1 h at RT, the plate was rinsed and 100 μl of rabbit anti-SBL at 1 μg/ml in PBS was added for 2 to 5 h. Protein A-peroxidase conjugate (Sigma P-8651) at 1 μg/ml was added and incubated for 2 h, after which the bound peroxidase activity was assayed with ABTS substrate 2,2′azino-di-(3-ethylbenzthiazoline sulfonic acid; Sigma A-1888). After color development, 50 μl of 0.06% NaN_3 was added to stop the reaction which was then monitored at 414 nm with a Flow Titertek Multiscan. A range of SBL concentrations from 50 to 0.015 ng was used as standards and the sensitivity of the assay was 0.4 ng. Since 50 to 100 μl of extract were usually applied, the sensitivity of the assay was 4 ng/ml.

RESULTS

Localization of Seed Proteins in Le$^+$ and Le$^-$ Cotyledons. Our first goal was to ascertain whether our antibodies to three different seed protein bind to cells of Le$^+$ cotyledons. We could then compare the localization patterns with those seen in Le$^-$ varieties. Figure 1 shows that SBL, KTi, and glycinin are located in protein bodies of cotyledon cells. No specific staining is seen when Le$^+$ cotyledon sections are incubated with nonimmune antibody (panel D). Many protein bodies are packed into these cells, as indicated in phase contrast sections (Fig. 2D). However, not all of the protein bodies react with each antibody, and many remain nonfluorescent. Furthermore, in those protein bodies which bind antibody, the distribution of fluorescence varies, with some staining only at their rims (denoted by arrows in Fig. 1, A and B) and many others exhibiting uniform fluorescence. The reason for these differences is not apparent, although incomplete penetration by the antibodies is possible. In addition, it is unclear whether individual protein bodies bind antibodies to all three proteins.

When seed of three Le$^-$ cultivars are examined, anti-glycinin and anti-KTi antibodies bind to cotyledon cells in a manner similar to that seen in Le$^+$. However, no reaction is seen with anti-SBL antibody (Fig. 2).

Localization of Antigenically Similar Proteins in Le$^+$ and Le$^-$ Roots. The above results show that anti-SBL binds to Le$^+$ cotyledon cells, but not those of Le$^-$. Thus, the antibody behaves in a manner consistent with other data on the presence of SBL in these lines (10, 21, 22). Furthermore, the fidelity of these results is reinforced by the staining observed for the other two proteins, glycinin and KTi, which should be present in Le$^-$ as well as Le$^+$. We then asked whether SBL or related proteins could be identified in other tissues of these soybean lines. A positive reaction is clearly evident in root sections of 4-d old Le$^+$ seedlings treated with anti-SBL (Fig. 3). Fluorescence is restricted to the outer epidermal layer of the roots, and as with cotyledon cells, it is distinctly particulate. The appearance and variable size of the particles and their similarity to lectin-containing vacuoles in

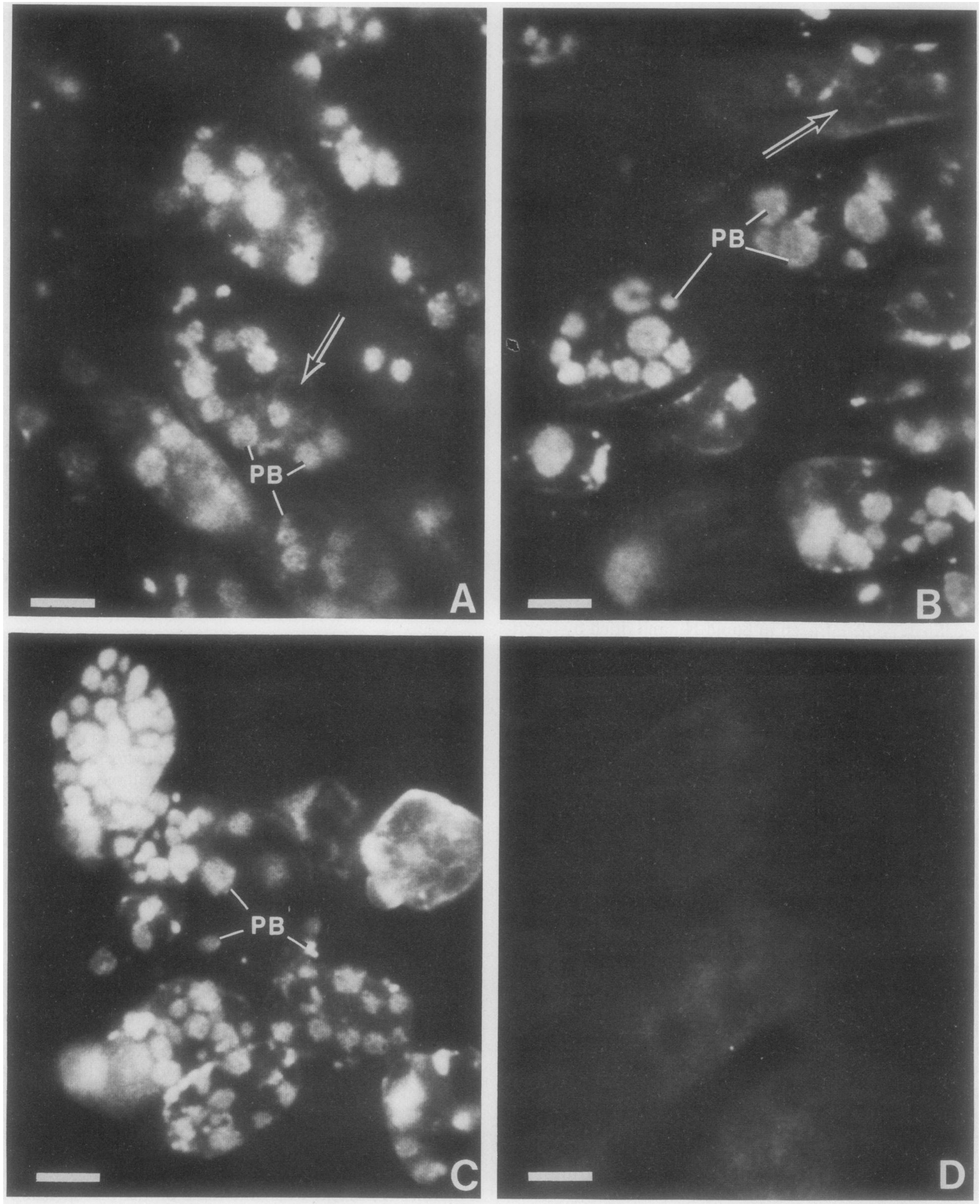

FIG. 1. Immunofluorescent localization of seed proteins in cotyledon sections from imbibed soybean seed of an Le+ variety (Williams). Monospecific rabbit antibodies to seed lectin (A), Kunitz trypsin inhibitor (B), glycinin (C), nonimmune rabbit immunoglobulin (D) were incubated with tissue sections and detected with rodamine-conjugated goat anti-rabbit antibody. All three proteins are co-localized in the protein bodies (PB) of the cotyledon cells. Bars = 10 μm.

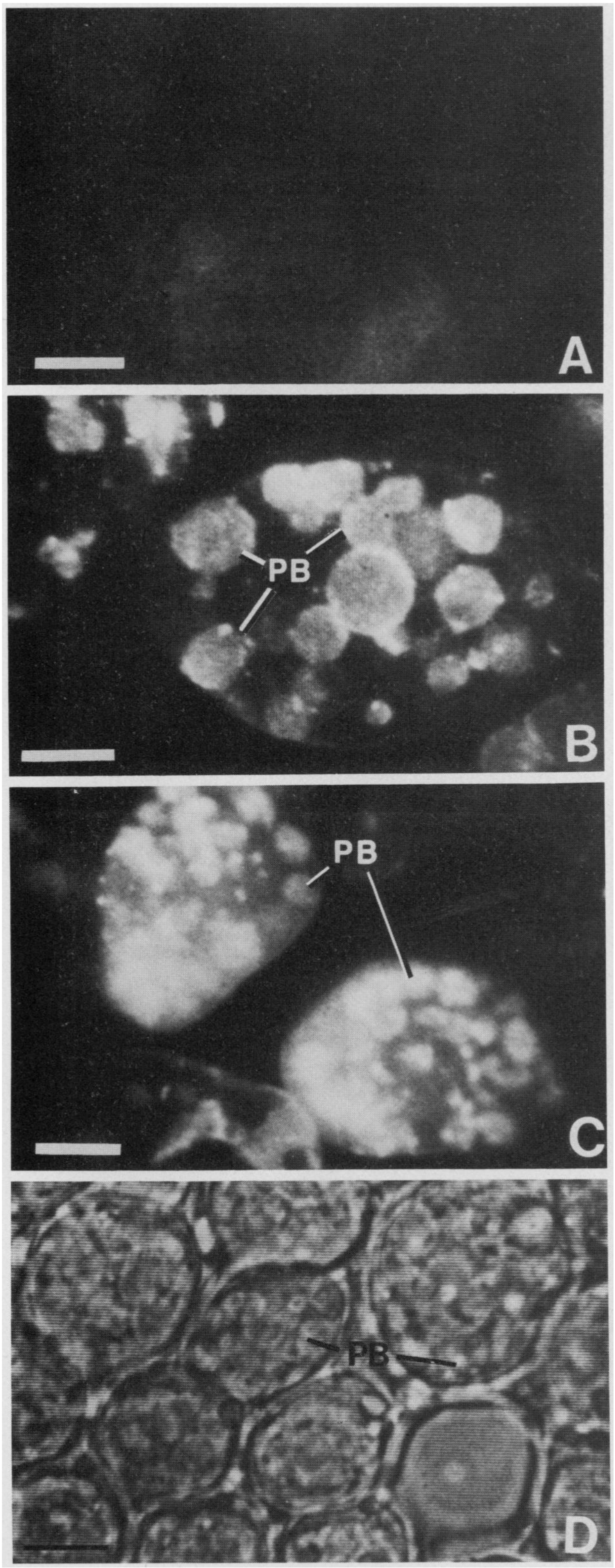

FIG. 2. Lectin is absent from the protein bodies of the Le⁻ line. Cotyledon sections from imbibed seed of an Le⁻ variety, Sooty, were examined by immunofluorescence using antibodies to lectin (A), trypsin inhibitor (B), or glycinin (C). Panel D is a phase contrast section showing the outline of individual cells and protein bodies (PB). Bars = 10 μm.

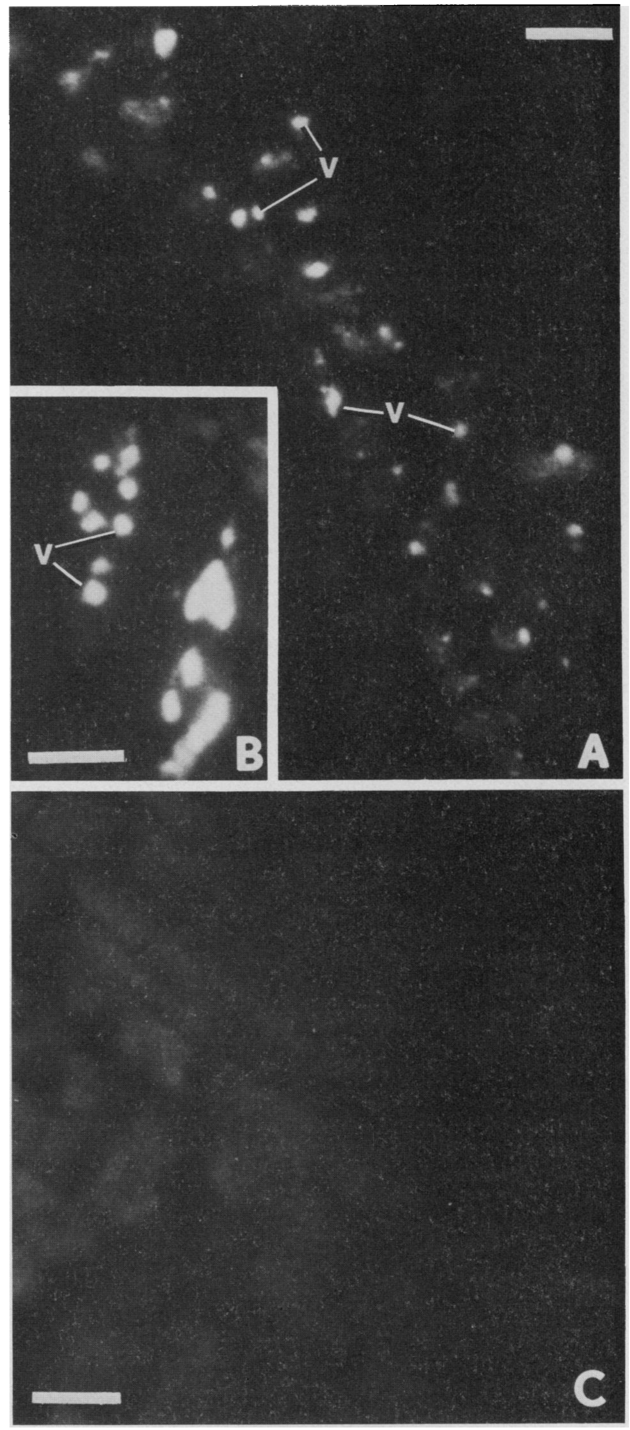

FIG. 3. Detection of a protein in the root tip cells of an Le⁺ variety (Williams) using antibody to seed lectin. Sections of 10 μm from the primary or lateral root tips of seedlings germinated for 4 d were treated with lectin antibody (A, B) or with nonimmune control immunoglobulin (C). V, vacuole; bars = 10 μm.

wheat roots (17) leads us to suggest that they are also vacuoles. When lateral and primary root tips of the Le⁻ seedlings are examined, they display similar anti-SBL fluorescence as well (Fig. 4). Attempts were made to determine whether a portion of the antibody binding material is surface located by incubating whole root hair slices in anti-SBL. While some preparations did appear to be positive (data not shown), the large amount of

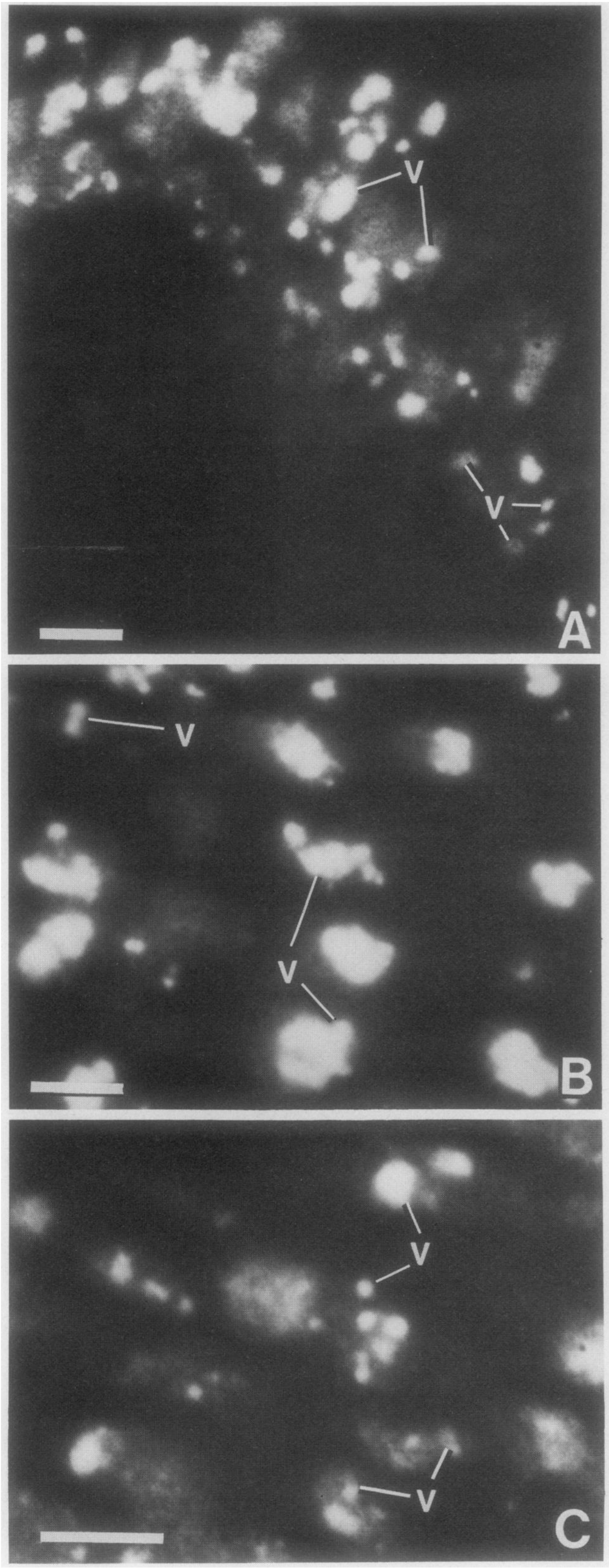

FIG. 4. A lectin CRM is also localized in root tip sections from Le⁻ varieties which are negative for the seed lectin. As in Figure 3, 10 μm sections were taken from the primary or lateral root tips of 4-d old seedlings. The Le⁻ soybean varieties and antibodies used were Sooty with lectin antibody (A), PI 171428 with lectin antibody (B), PI 90768 with trypsin inhibitor antibody (C). Controls of each variety with nonimmune immunoglobulin resembled the control shown in Figure 3C. V, Vacuole; bars = 10 μm.

background autofluorescence in soybean root hairs prohibited a definitive determination.

Anti-KTi antibody gave a weak response in most cases, but there was clear binding of the antibody by the root epidermal cells in one of the lines used, PI 90768 (Fig. 4C). There was essentially no detectable localization of product with the glycinin antibody in roots of any of the four soybean lines examined (data not shown).

Immunoblotting and ELISA Experiments. To determine the nature of the root protein which cross-reacts with antibody to seed lectin, extracts were analyzed on Western blots following SDS-PAGE. The roots of both Le^+ and Le^- seeds contain a band of 33 kD which is detected by anti-SBL as shown in Figure 5. The root CRM is slightly larger than the 30 kD polypeptide which represents SBL subunits. The seed lectin often appears as a set of 3 to 4 closely spaced bands even when phenylmethylsulfonyl fluoride is included in the extracts as a protease inhibitor. This slight heterogeneity could result from small diferences in glycosylation of the subunits. Smaller mol wt fragments of 19 and 16 kD appear in some cotyledon extracts and are also obtained in preparations of lectin which have been purified by affinity chromatography. These are likely to be specific degradation fragments of the 30 kD subunit since these fragments are not detected in Le^- cotyledons (Fig. 5).

Besides its presence in roots, the 33 kD polypeptide is also found in embryo axes (Table I) and in leaves of seedlings and older plants (Fig. 6). In addition, a polypeptide with a mol wt of 26 kD is present in leaves as well. This polypeptide is not detectable in the initial PBS supernatant of leaf extracts but appears when the pellet is subjected to SDS and boiling. Increased amounts of the 33 kD protein could be obtained if root pellets were also subjected to the same procedure (Fig. 6).

To quantitate the relative amounts of lectin and cross-reacting protein in seeds and roots, SBL standards were immunoblotted and band intensities compared with those of the extracts. SBL is

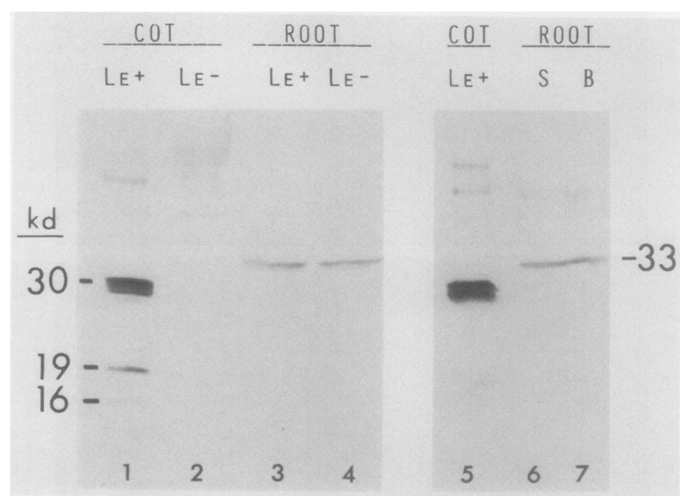

FIG. 5. Immunoblots comparing the lectin found in seed and root tissues. Extracts from dry cotyledons (COT) or from root tips of 4-d seedlings were prepared as described under "Materials and Methods." The proteins were separated on SDS-polyacrylamide gels, blotted to nitrocellulose and detected with antibody to seed lectin and alkaline phosphatase conjugated second antibody. Unless otherwise noted, 20 μl of undiluted extracts were applied to the sample wells. Sizes are indicated in kD. Lane 1, an Le^+ cotyledon sample (Williams, 20 μl of a 1/200 dilution); lane 2, an Le^- cotyledon sample (Sooty); lane 3, an Le^+ root extract (Williams); lane 4, an Le^- root extract (Sooty); lane 5, same as lane 1; lane 6, the soluble (S) supernatant from an Le^- root extract (Sooty); lane 7, the 'bound' (B) material released from the root extract pellet by 1% SDS and boiling.

Table I. *Relative Amounts of Seed Lectin (30 kD, SBL Polypeptides) or Anti-SBL Cross-Reacting Material (33 kD, CRM Polypeptide) in Le⁺ and Le⁻ Plant Parts as Determined by ELISA and Western Blotting*

Values are expressed in ng per single seed for the cotyledons and axes of mature grain and for individual primary root tips of 4-d old seedlings. Several independent determinations for each assay were made. Quantitations for both bands on Western blots were estimated against purified SBL standard concentrations ranging from 0.3 to 100 ng. ND = not detectable at the sensitivity in ng per plant part shown in parentheses.

	Cotyledons[a]		Axis		Primary Root Tip[b]	
Soybean line[c]	Le⁺	Le⁻	Le⁺	Le⁻	Le⁺	Le⁻
ELISA	1×10^6	ND	500	ND	ND	ND
	(20)	(20)	(4)	(4)	(0.05)	(0.05)
30 kD SBL (Western)	4×10^6	ND	2000	ND	ND	ND
	(150)	(150)	(30)	(30)	(0.18)	(0.18)
33 kD CRM (Western)	ND	ND	500	500	2.0	2.0
	(150)	(150)	(30)	(30)	(0.18)	(0.18)

[a] Represents both cotyledons of a single seed. [b] Pooled extracts of root tips were made as described in "Materials and Methods." Sensitivities per root tip are calculated in the following manner for the ELISA assay in which the detection limit was 0.4 ng in a 50 μl assay of an extract of approximately 100 root tips in 600 μl total volume ($0.4 \times 12 \div 100 = 0.05$). In the Western blots, the detection limit was 0.6 ng in a 20 μl assay of an extract of approximately 100 root tips in 600 μl total volume ($0.6 \times 30 \div 100 = 0.18$). [c] The Le⁺ line used was Williams and the Le⁻ line was Sooty for all determinations.

present at a level of 4 mg in the cotyledons of individual seed and 2000 ng per embryo axis in the Le⁺ cultivar, whereas no seed lectin is detected in undiluted extracts of Le⁻ cotyledons or axes from individual seeds (Table I; Fig. 5). ELISA was also used to quantitate the amount of lectin in the seed and axes and the values were about 4-fold lower than those estimated by quantitative Western blots. However, the absolute sensitivity per plant part in the ELISA is better than that of the Western procedure primarily because a larger sample volume could be analyzed (100 μl for the ELISA *versus* 20 per well for blotting). Thus, the cotyledons and axes of Le⁻ cultivar are found to be negative for SBL to a sensitivity of 20 and 4 ng per plant part, respectively, as determined by ELISA (Table I). While one report has indicated that hemagglutination activity could be found at very low levels in the seed of several Le⁻ cultivars, including Sooty (28), our results indicate that no SBL polypeptides can be found. However, it is known that several other lectin activities are found in soybean seed, some of which are also present in the SBL negative lines (2, 5, 7).

In contrast to the moderate to high levels of the 30 kD SBL subunits in embryo axes and cotyledons of Le⁺ varieties, much lower amounts of the 33 kD protein are present in roots. As determined by immunoblotting, approximately 2 ng per root tip are found. Since quantitations were done on pooled extracts of primary root tips (see "Materials and Methods") or on small volume extracts of individual primary or lateral root tips and not on whole root tissue, extrapolation to a value of about 20 ng per seedling roots of a 4-d plant is only a rough approximation. Additionally, these estimates must be taken as minimal levels since the 33 kD protein may be reacting with only a certain percentage of the antibodies produced against seed lectin subunits.

ELISA assays on root tip extracts were not reliable because of the very low amounts present and because of the sporadic presence of background readings in the concentrated samples used. Western blots revealed the presence of the 33 kD band in the axes of Le⁻ lines at moderate levels despite the fact that no cross-reaction was detected to a sensitivity of 4 ng per axis by

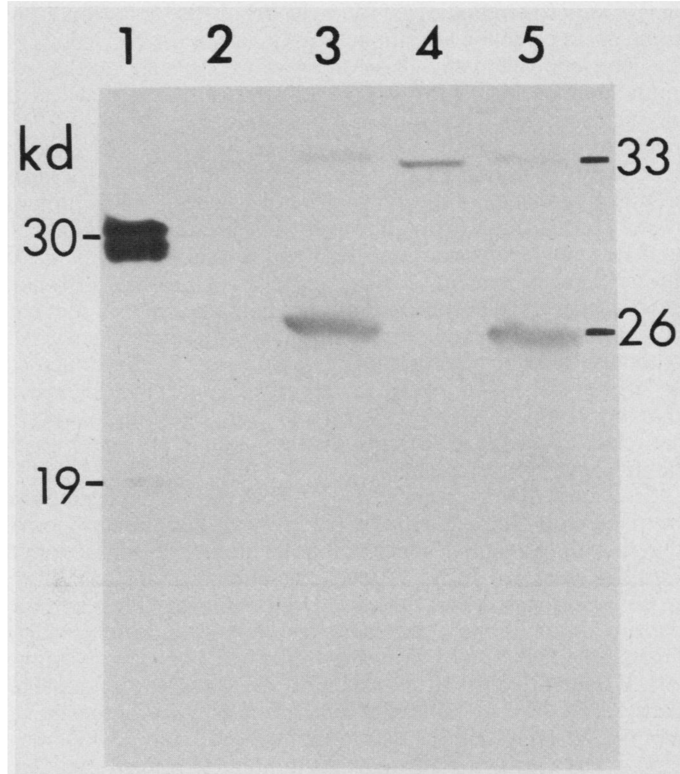

FIG. 6. Presence of a lectin CRM in leaves. Extracts from leaves of 4-d or adult leaves were separated on SDS-polyacrylamide gels, electroblotted to nitrocellulose, and detected with antibody to seed lectin and alkaline phosphatase conjugated second antibody. Twenty μl of the soluble supernatant or of the bound material released by treatment of the pellet with 1% SDS and boiling was loaded onto the sample wells. Sizes are indicated in kD. Lane 1, an Le⁺ cotyledon sample (Williams, 20 μl of a 1/100 dilution); lane 2 (soluble) and lane 3 (bound), extracts from leaves of an Le⁺ variety (Williams); lane 4 (soluble) and lane 5 (bound), extracts from leaves of an Le⁻ variety (Sooty).

ELISA (Table I). It is likely that the capture antibody used in the sandwich assay (antibody raised in goats to native SBL) does not recognize the 33 kD band; therefore, our ELISA will not detect this protein in axes or roots. Similarly, we have also noted that our preparation of rabbit antibody made against native, tetrameric SBL was much less sensitive in detecting denatured SBL subunits from SDS gel blots than was our antibody lot (also from rabbits) made in response to SBL subunits prepared with 0.1% SDS before injections.

DISCUSSION

Several reports have now identified lectins (or related proteins) in plant roots including those of nonlegumes (1, 4, 6, 14, 23, 25). Our results show that a protein which binds anti-SBL antibody is located in the roots of four soybean varieties, regardless of whether SBL is present in the seed. The root protein can be visualized by two sensitive assays, *in situ* immnocytochemistry and *in vitro* immunoblotting. The latter procedure shows that the SBL-like root protein is present in ng/plant quantities, compared to the mg levels of SBL found in seeds.

Our confidence in these results is bolstered by several other observations. First, lectin was clearly identified in the Le⁺ cotyledons using both procedures and was confirmed by ELISA using the same antibody. Control experiments with nonimmune antibody yielded no specific reaction. Previous studies have shown that the seed lectins of legumes including soybean are located in cotyledon protein bodies (3, 12; reviewed in Ref. 6). Second, the same antibody failed to stain protein bodies in the cotyledons of Le^- cotyledons and this observation was confirmed by the immunoblotting and ELISA assays. Lectin has not been detected in such varieties, including those examined here, by affinity chromatography and radioimmunoassay (21, 22). The lack of seed lectin in the Sooty cultivar is known to be due to a large insertion sequence in the single gene that codes for this protein (10, 31). It is noteworthy, however, that protein bodies in Le^+ and Le^- cotyledons bind anti-KTi and anti-Gly. Both of these proteins are present in all three cultivars. Third, the SBL-like protein appears to be localized in vesiculate structures in the root epidermis, and its extraction is amplified by denaturing agents. Although these structures are not as uniform in appearance or as large as cotyledon protein bodies, their appearance indicates that they could be vacuoles. Because protein bodies and vacuoles are related organelles, SBL and the root protein are localized in homologous compartments.

Gade *et al.* (8) have isolated a GalNAc-sensitive hemagglutinin from the roots of an Le^+ cultivar (Chippewa). They also proposed that this lectin, some of which reappears on the root surface after washing, is stored in an intracellular compartment (9). More recently, Halverson and Stacey (11) have shown that root exudates of both Le^+ and Le^- varieties contain a protein which facilitates nodulation by *Rhizobium* in a GalNAc-sensitive manner. While our observations that anti-SBL binds to vacuole-like particles on the root epidermal cells, is consistent with the above reports, we have not yet determined whether the 33 kD root protein has a lectin activity which is inhibitable by GalNAc. Our attempts to purify this protein on GalNAc affinity columns from root extracts of an Le^- variety have been unsuccessful to date. Since the report by Gade *et al.* (8, 9) did not include a Western blot analysis of the protein which they purified from roots of the Le^+ variety, we cannot distinguish whether they have purified the 33 kD band, another root protein with GalNAc activity, or minute contaminant amounts of SBL from the cotyledons. It is known that SBL can leach from the cotyledons during seed imbibition.

A role for lectins in the interaction between legume roots and rhizobial symbionts has been proposed (reviewed in Dazzo and Gardiol [4]). Although the binding of bacteria to root cells via lectin intermediates has been reported in clover (4), similar activity in soybean has been the subject of debate (11, 26, 28). In particular, the nodulation of cultivars which lack seed lectin (and presumably a root counterpart as well) seemed to mitigate against a role for these proteins in symbiont attachment (22). Our work demonstrates that SBL is not present in the seeds or roots of the Le^- varieties but an antigenically related protein is present in the roots. Therefore, this result and other reports on the occurrence of lectin-like activities in soybean roots do not rule out that proteins other than SBL could potentially be involved in some phase of Rhizobial recognition.

Root lectins have been reported in other legumes including pea and bean (1, 14, 23), but a protein similar to seed lectin is not present in the roots of *Dolichos biflorus*, although one does occur in leaves and stems (6, 27). The 33 kD protein in soybean is also present in leaves, along with a 26 kD anti-SBL binding moiety. The latter is only extracted under denaturing conditions and therefore may be tightly associated with membranes or the cell wall. A significant portion of the *Dolichos* leaf protein is reported to be associated with the cell wall (6, 27). The function of leaf lectins remains obscure, but an involvement in microbial recognition must be considered as well.

It is interesting that the Kunitz trypsin inhibitor or a related protein is also present in roots. Although lectin and trypsin inhibitor are clearly seed entities, their presence in roots could signify functions common to both vacuoles and protein bodies. It is not clear why the KTi and SBL-like polypeptides appear to be restricted to the root epidermis and are not found in other root tissues.

Some nonseed lectins share immunological and biochemical characteristics with their seed counterparts in the same plant, but differences have also been identified (6, 14, 17, 23). These differences could be due to posttranslational modifications in the product by a single gene or to the expression of separate genes in various tissues and organs. Our evidence srongly indicates that the 33 kD polypeptide in soybean roots represents a gene product coded by a locus other than *Le1* which encodes the 30 kD seed lectin subunit. Our analyses show that SBL is not detectable in the cotyledons of three Le^- lines. In one of these lines, Sooty, the level of SBL mRNA is reduced 10,000-fold compared to Le^+ seeds due to the insertion of a transposable element in the coding region of the gene (10, 31). Thus, the antigenically related root protein must be designated by a different DNA sequence. Southern blotting experiments show that another DNA fragment of interest is present in both Le^+ and Le^- varieties (10). Designated *Le2*, this gene shows 85% homology at the nucleotide level with *Le1* (L Vodkin, P Rhodes, unpublished data). It is not yet clear whether *Le2* is expressed or is a pseudogene. However, it does not appear that the *Le2* gene encodes the root 33 kD protein as no mRNAs complementary to either the *Le1* or *Le2* genes have been found in S1 hybridization experiments using total root mRNA from 4-d old seedlings of Le^+ or Le^- varieties (L Vodkin, unpublished data). However, in Le^+, but not Le^- plants, a very low level of mRNA complementary to the *Le1* seed lectin gene has been found in mRNA preparations from roots of adult soybean plants (J Okamura, R Goldberg, personal communication). The 33 kD polypeptide could be coded by a gene more remotely related to that of the seed lectin than *Le2*. Such a gene might have lower nucleotide homology and not be observed in DNA blots using the *Le1* sequence as a probe. A similar consideration pertains to the 26 kD protein in leaves.

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